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CRITICAL EXPERIMENTS TO DETERMINE FEASIBILITY OF ORGANIC CONTAMINANT BIODEGRADATION IN CATIONIC SURFACTANT TREATED MEDIA

Ronald J. Spanggord, G. Ross Gordon,
Kristien E. Mortelmans

SRI International
333 Ravenswood Avenue
Menlo Park CA 94025

ENVIRONICS DIRECTORATE
139 Barnes Drive, Suite 2
Tyndall AFB FL 32403-5323

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JEFFREY STINSON, Capt, USAF
Project Officer

ALLAN M. WEINER, Lt Col, USAF
Chief, Risk Management Division

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Ronald J. Spanggord, G. Ross Gordon and Kristien E. Mortelmans

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SRI International
333 Ravenswood Ave.
Menlo Park, CA 94025

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A soil column with 20 sampling ports was designed to assess the binding and potential microbial degradation of naphthalene in Columbus Air Force Base aquifer soil treated with the surfactant, hexadecyltrimethylammonium bromide (HDTMA). HDTMA was found to be toxic to most aerobic soil microorganisms when applied to the soil. However, when the HDTMA was bound to soil, the toxicity decreased and the microbial populations readily recovered to normal levels. Naphthalene was used as a test substrate and was found to readily bind to HDTMA treated soil under anaerobic conditions. When aeration was applied, microbial populations adapted to HDTMA at a faster rate than to the test substrate, naphthalene. This phenomenon caused naphthalene to be desorbed from the soil and naphthalene began to migrate behind metabolized surfactant. Naphthalene metabolism was not observed as assessed by chromatographic analysis of soil sample extracts collected along the column length. Metabolized surfactant was found to leach from the soil column. Under the conditions of this study, it appears that the surfactant technology may have limited utility in soils where pollutant-adapted microbial populations do not recover at sufficient speed to compete with surfactant utilization.

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PREFACE

This report was prepared by SRI International, 333 Ravenswood Ave, Menlo Park, CA 94025, Contract No. F08635-93-C-0020, for Applied Research Associates, 4300 San Mateo Blvd., Suite A-200, Albuquerque, NM 87110 and the Armstrong Laboratory Environics Directorate (AL/EQ), 139 Barnes Drive, Suite 2, Tyndall Air Force Base, Florida 32403-5323.

This final report describes an evaluation of the toxicity of the soil surfactant, hexadecyltrimethylammonium bromide (HDTMA), to soil microbial populations in an aquifer soil column, the sorption of a test pollutant (naphthalene) to HDTMA-treated soil, and the construction of a laboratory soil column to evaluate the movement and biotransformation of naphthalene under anaerobic and aerobic conditions. The results of this study provide an assessment for the loss and movement of pollutants in HDTMA-treated soils.

This work was performed between May 1995 and March 1996. The ALEQW project officer was Captain Jeffery Stinson, USAF. The Applied Research project manager was Dr. Raymond Montgomery.

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EXECUTIVE SUMMARY

A. OBJECTIVE

The purpose of this investigation was to determine the potential for the microbial degradation of naphthalene to occur when sorbed to soil treated with the cationic surfactant, hexadecyltrimethylammonium bromide (HDTMA).

B. BACKGROUND

The treatment of soil with a cationic surfactant is an emerging technology that can be used to retard the movement of pollutants in soil by increasing the soil's sorption capacity. Cations, such as HDTMA, bind strongly to the anionic portion of soil to effectively increase the organic carbon loading of the soil. When this occurs, organic pollutants can become more strongly sorbed to the soil and their migration to groundwater is retarded.

C. SCOPE

The use of surfactants in soils to retard pollutant migration has the potential to be a viable soil remediation technique. The technology, however, has not been evaluated in a soil column that more closely approximates an environmental scenario. In this study, a soil column was constructed to evaluate the loss and movement of naphthalene in a surfactant-treated soil. Studies were conducted to assess the toxicity and recovery of soil microorganisms in a soil column of surfactant-treated soil, the increase in binding of naphthalene on surfactant-treated soil, and the metabolic disposition of naphthalene when subjected to anaerobic and aerobic conditions in the soil column. The utility of using this technology to retard the movement of pollutants in soil is discussed.

D. RESULTS AND CONCLUSIONS

The utility of this technology would be enhanced if biotransformation of the pollutant occurred in the sorbed state. A soil column with 20 sampling ports was designed to assess the binding and potential microbial degradation of naphthalene in Columbus Air Force Base aquifer soil treated with the surfactant, HDTMA. HDTMA was found to be toxic to most aerobic soil microorganisms when applied to the soil. However, when the HDTMA was bound to soil, the toxicity decreased and the microbial populations readily recovered to normal levels.

Naphthalene was used as a test substrate and was found to readily bind to HDTMA-treated soil under anaerobic conditions. When aeration was applied, microbial populations adapted to HDTMA at a faster rate than to the test substrate, naphthalene. This phenomenon caused naphthalene to be desorbed from the soil and naphthalene began to migrate behind metabolized surfactant. Naphthalene metabolism was not observed as assessed by chromatographic analysis of soil sample extracts collected along the column length. Metabolized surfactant was found to leach from the soil column. Under the conditions of this study, it appears that the surfactant technology may have limited utility in soils where pollutant-adapted microbial populations do not recover at sufficient speed to compete with surfactant utilization.

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SECTION I

INTRODUCTION

A. OBJECTIVE

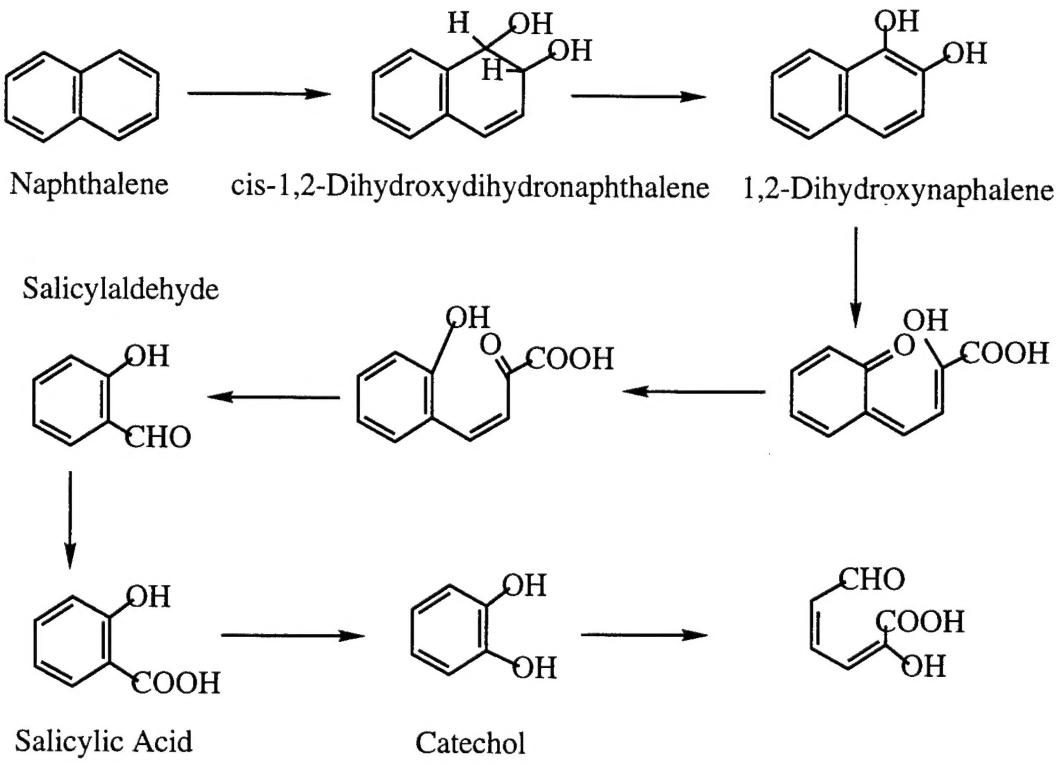
The objective of this study was to design a laboratory soil column and study the potential for the biotransformation of a sorbed pollutant (naphthalene) on a Columbus Air Force Base soil treated with the surfactant, hexadecyltrimethylammonium bromide, under both anaerobic and aerobic conditions.

B. BACKGROUND

The application of cationic surfactants to soil offers technology for soil modification to provide a sorptive zone that will inhibit the migration of selected organic contaminants to ground waters (Burris and Antworth, 1992). Cationic surfactants bind readily to the cation exchange capacity (CEC) sites in soil and effectively increase the carbon-loading of the soil. This increase in soil sorption capacity can retard chemical migration by several orders of magnitude (Lee et al., 1989). The usefulness of this technology would be greatly increased if biotransformation of the sorbed organics was demonstrated.

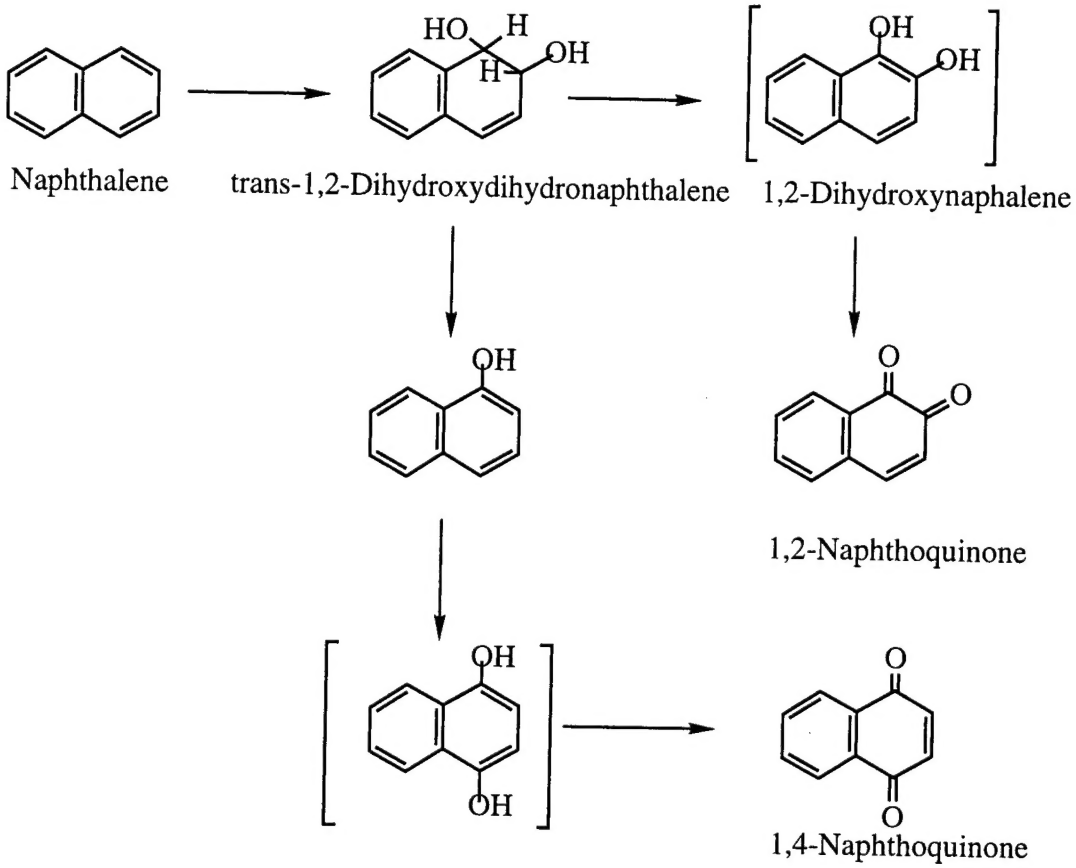
Hexadecyltrimethylammonium bromide (HDTMA) is a cationic surfactant that binds readily to soils. It is also highly toxic to soil microorganisms by binding to cellular membranes to disrupt cellular function (Denyer and Hugo, 1991). However, once bound to soil, the toxicity of HDTMA is significantly reduced and microbial viability can return to the soil (Nye et. al, 1994). Extensive studies have been performed by Dr. Steven Boyd and his colleagues at Michigan State University. Their findings show that it becomes feasible to enhance soil sorption of organics and couple sorption to *in situ* biodegradation. This combined technology would offer a feasible alternative in soil treatment remediation.

To assess naphthalene biodegradation, we need to understand pathways and products that might be derived from naphthalene by soil bacteria. The pathway has been well-studied; however, little is known about the metabolism of naphthalene in experimental systems designed to model natural ecosystems. Davies and Evans (1964) proposed and partially verified an oxidative pathway for naphthalene metabolism by soil pseudomonads shown below.



The initial step involves molecular oxygen and a dioxygenase enzyme to form the cis-1,2-dihydroxydihydronaphthalene (Ensley et al., 1982). Davies and Evans (1964) confirmed a number of the following steps using cell-free enzyme preparations from soil pseudomonads grown on naphthalene. Further work by Cerniglia et al. (1980) confirmed a number of the metabolites produced by bacteria.

Fungi can also metabolize naphthalene in a manner similar to mammalian systems and produce 1,4-naphthoquinone and 1,2-naphthoquinone as additional potential metabolites in soil (Cerniglia and Gibson, 1977). This pathway is shown below.



The physical, chemical, and microbial properties of soil can vary greatly and these properties will affect the disposition and persistence of chemicals in the environment. By understanding the potential products that could result from naphthalene, we can assess the effect of surfactant on naphthalene metabolism and, therefore, its disposition in soil.

C. SCOPE

The majority of the work by the Michigan State University researchers has been performed in shaker flask studies, but the applicability of the technology to a laboratory soil column has not been studied extensively except for the investigations conducted by Burris and Antworth (1992). The scope of this study included (1) an assessment the toxicity of HDTMA to soil bacteria in a soil column, (2) a determination of the enhanced soil sorption of a test chemical (naphthalene) to HDTMA-treated soil compared to a control, and (3) the design a soil column for evaluating naphthalene biodegradation and sorption to HDTMA-treated soil.

SECTION II

METHODS

A. SOIL

Soil obtained from Columbus Air Force Base through Captain Jeffery Stinson was received on July 8, 1995. This soil was identified as a burrow pit aquifer soil. Large rocks and debris were removed from the bulk of the soil by sieving through a 2-mm sieve. The soil was tested for texture classification, pH, cation exchange capacity (CEC), organic carbon content, bulk density, cation content, and moisture holding capacity. The determinations were performed by Agvise Laboratories, Northwood, ND. The results appear in Figure 1.

B. SOIL COLUMN TOXICITY EVALUATION

1. Preparation of HDTMA-Treated Soil.

Bulk soil was broken up into large lumps, dried overnight at 40°C, then sieved through a 2-mm screen. Three samples of about 50 grams each of the sieved soil were dried for 15 hours at 96°C and the moisture loss was calculated to be 3.5%. The dried soil was treated with HDTMA (Aldrich Chemical Company) at a rate that was four times the reported cation exchange capacity of 6.0 meq/100 grams. (See soil test report, Figure 1). To 54.6 grams of the dried soil was added 50 mL water and 4.8 grams of HDTMA in a 250 mL polypropylene screw-cap bottle. The contents were magnetically stirred 4 hours at room temperature and the sample centrifuged 30 minutes at 4000g in a cold room at 4°C. The supernatant liquid was decanted, 50 mL water was added, the bottle was briefly shaken to resuspend the soil, and the suspension was centrifuged. After the seventh wash, the bottle was lyophilized overnight to yield a free-flowing powder.

2. Soil Activation

Soil microorganisms were activated by placing approximately 1000 grams of soil in a wide mouth screw-cap polypropylene jar followed by addition of water at a level of 75% of the soil field capacity (12.2 grams/100 grams soil). The jar was left uncapped and exposed to the air for 5 days. Weight loss was monitored daily and the loss was added back as water. No other supplemental nutrients were provided.

3. Soil Toxicity Tests

Polypropylene syringe barrels (10 mL) were plugged with glass wool and then lightly packed with 15 grams of activated soil to the 10 mL mark. A stock solution of HDTMA (0.034 meq/mL) in water was added at a rate of 0%, 10%, 20%, 40%, and 80% of the soil CEC of 6.0

AGVISE LABORATORIES

P.O. BOX 510, NORTHWOOD, ND 58267
(701) 587-6010

SOIL TEST REPORT

FIELD BURROW PIT SAMPLE COL AFB
COUNTY
TWP SECTION
QTR ACRES
PREV CROP

N
W E
S

SUBMITTED FOR:

DR. RONALD SPANGGORD
SRI INTERNATIONAL
333 RAVENSWOOD AVE
MENLO PARK CA 94025

SUBMITTED BY: SR1112

SRI INTERNATIONAL
333 RAVENSWOOD AVE
MENLO PARK CA 94025

REF # 2461290
LAB # 10428 BOX # 1992

DATE SAMPLED DATE RECEIVED 7/18/95 DATE REPORTED 7/24/95

NUTRIENT IN THE SOIL		INTERPRETATION				1ST CROP CHOICE			2ND CROP CHOICE			3RD CROP CHOICE		
		V LOW	LOW	MED	HIGH									
Nitrate N						YIELD GOAL			YIELD GOAL			YIELD GOAL		
						SUGGESTED GUIDELINES			SUGGESTED GUIDELINES			SUGGESTED GUIDELINES		
						LB /ACRE		APPLICATION	LB /ACRE		APPLICATION	LB /ACRE		APPLICATION
						N			N			N		
Phosphorus						P_2O_5			P_2O_5			P_2O_5		
Potassium	37 ppm	******				K_2O			K_2O			K_2O		
Chloride						Cl			Cl			Cl		
Sulfur						S			S			S		
Boron						B			B			B		
Zinc						Zn			Zn			Zn		
Iron						Fe			Fe			Fe		
Manganese						Mn			Mn			Mn		
Copper						Cu			Cu			Cu		
Magnesium	110 ppm	********				Mg			Mg			Mg		
Calcium	900 ppm	**********				Lime			Lime			Lime		
Sodium	112 ppm	**********												
Organic Matter	1.7 %	*******												
Soluble Salts														

Soil pH	Buffer pH	Cation Exchange Capacity	% Base Saturation (Typical Range)				
			% Ca	% Mg	% K	% Na	% H
6.3		6.0 meq	(65-75) 75.0	(15-20) 15.3	(1-7) 1.6	(0-5) 8.1	(0-5)

SOIL TEXTURE: SAND: 70%; SILT: 20%; CLAY: 10% SOIL CLASSIFICATION: SANDY LOAM
1/3 BAR MOISTURE = 12.2 % BULK DENSITY = 1.17

CAM-7524-1

Figure 1. Agvise Laboratories soil classification report for Columbus AFB aquifer soil.

meq/100 grams (equivalent to 2.7, 5.3, 10.6, and 21.2 mL of HDTMA, respectively). To maintain equivalent volumes through the columns, the lower concentrations of HDTMA were diluted with enough water to equal the volume passed through the column receiving the highest quantity of HDTMA. A pressure head was generated by suspending the HDTMA in empty syringe barrels approximately 2-1/2 to 3 feet above the soil columns and connected with 2-3-mm i d Tygon tubing. Approximately 16 to 24 hours were required for the HDTMA to flow through the columns. Then the columns were washed with 20 mL of water in a similar manner. The bottom of the plunger was cut off and the soil column extruded onto a sheet of weighing paper and sliced into 4 approximately equal sections that were placed in scintillation vials for total microbial viability counts.

4. Procedure for Determining Viable Microbial Counts in Soil Samples.

Approximately 1-gram samples were provided in glass liquid scintillation vials for microbial counts. The net weight of each sample was provided and was used to determine the volume of sterile phosphate buffered saline (PBS) needed to yield the weight of the soil to be equivalent to 1 gram in 10 mL. This ratio of soil to PBS made it possible to disperse the soil adequately by vortexing to make a suspension that could be readily transferred by a 1-mL pipette. This stock suspension represented the 10^{-1} dilution and was left at room temperature for about 30 minutes with frequent vortexing. From this dilution, five additional 10-fold dilutions were made by sequentially transferring 0.5 mL of each dilution in 4.5 mL of sterile PBS.

Aliquots of either 0.01 or 0.1 mL were transferred to trypticase soy plates in triplicate. The 0.1-mL samples were aseptically spread over the surface of the agar plates, using an alcohol-flamed glass rod. The plates were left at room temperature for about 2 days then were examined by using a dissecting microscope. The low incubation temperature prevented molds and fungi from overgrowing the plate and yielded distinct bacterial colonies that were readily observed microscopically. The results were expressed as colony forming units/mL (or per gram) of soil.

On two occasions the colonies were categorized in terms of colony morphology only. The colonies most often encountered were about 1 mm or less in diameter and were presumed to represent the same organism. The presence of molds/fungi was noted on some plates, but their presence did not interfere with colony counting or the description of the colonies.

C. HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF NAPHTHALENE AND METABOLITES

The naphthalene used in this study was obtained from Baker Chemical Company. Obtained from Aldrich Chemical Company were catechol, 1,2-naphthoquinone, 1,4-naphthoquinone, 1,2-dihydroxynaphthalene, 1-naphthol, 2-naphthol, salicylaldehyde, and salicylic acid. 1,2-dihydroxydihydronaphthalene was a generous gift from Dr. David Gibson, University of Iowa. HPLC was performed using a Waters Model 600 controller, 717 WISP autoinjector, and a 996 diode array detector. All units were controlled using Water's Millennium software. The following conditions were employed:

Column: Alltech Altima C18, 5μ, 4.6 x 250 mm
Solvent: Acetonitrile/Water (30/70) hold 2 minutes -----> 70/30 at 6 minutes
Hold 70/30 to 20 minutes, then return to initial conditions
Detector: 200 - 600 nm @4.8 nm resolution

Retention Times:		
	Salicylic acid	2.083 minutes
	cis-1,2-Dihydroxydihydronaphthalene	4.808 minutes
	Catechol	5.168 minutes
	1,2-Naphthoquinone	10.220 minutes
	1,2-Dihydroxynaphthalene	11.452 minutes
	Salicylaldehyde	12.068 minutes
	1,4-Naphthoquinone	12.312 minutes
	2-Naphthol	12.860 minutes
	1-Naphthol	13.292 minutes
	Naphthalene	18.185 minutes

Components were identified by their retention time and UV spectrum contained within a project library.

D. NAPHTHALENE SOIL SORPTION STUDY

1. Binding of Naphthalene

Adsorption isotherms were determined by using the batch equilibration technique described by Lee et al. (1990). Stock solutions of naphthalene were prepared in methanol (1.6 and 51 mg/mL), and 5 to 75 μL aliquots were diluted to 50 mL with water to yield six concentrations that ranged from 1 to 20 ppm naphthalene. Tubes (16 x 150 mm) containing 100 mg of the HDTMA treated soil were essentially filled with 20 mL of the various naphthalene standards leaving a head space of about 0.2 mL. The tubes were sealed tightly with polypropylene caps, and placed in a horizontal position on a reciprocating shaker (~ 90 excursions/minute) for 24 hours at room temperature. All the sample handling was performed as quickly as possible to minimize losses due to the volatility of naphthalene.

2. HPLC Assay of Naphthalene

A 2-mL polypropylene microfuge tube was filled (no residual headspace) with the equilibrated naphthalene-soil sample water, sealed tightly, and centrifuged four minutes at 14,000g. The clarified supernatant liquid was quickly transferred to 300-μL microinjection vials and 50 μL were subjected to HPLC. Standards for HPLC were prepared by diluting the methanolic stock solutions of naphthalene with water to yield solutions containing to 20 ppm naphthalene. These samples were chromatographed immediately after the dilutions were prepared.

E. SOIL COLUMN STUDY

1. Preparation of the Soil Column

A soil column was fabricated from acrylic tubing measuring 6.5 cm by 30 cm. Ten holes were drilled on each side of the tube in a circular fashion and acrylic tubes (1.2 x 2.5 cm) were cemented into each hole. A funnel, containing a stopcock, was cemented to the bottom of the tube. The top the tube was closed with a rubber gasket between an acrylic plate and secured by six screws. The center of the plate contained a stainless steel metal fitting connected to a 1/16 inch tubing hex nut. Fluid was delivered to the column with the aid of an Isco Model 500D syringe pump. A schematic of the column is shown in Figure 2.

2. Packing and Sampling of Large Column

Glass wool was inserted from the inside into the funnel shaped end of the column (approximately 1/2-inch layer) and then the remaining funnel shape was filled with glass beads until the cylindrical portion of the column was reached. The glass beads were covered with a 1/4-inch layer of glass wool and then the conditioned soil was added and packed in the column in 2 to 3-cm increments with the aid of a 3/4-inch glass rod, tamping each addition firmly into a level segment. Each side port was filled with glass wool flush with the inside opening to the column and then the port was sealed with a rubber sleeve-type stopper. While under pressure, stoppers were held in place with wire or plastic wire ties placed over the ports and completely around the column. The column was packed with soil to about 1 cm above Sampling Port 1 with a remaining head space of about 50 mL for the addition of naphthalene.

Water (500-1000 mL) was deoxygenated in a screw-capped bottle by sparging with helium for 15 to 30 minutes. HDTMA was added to make a final concentration of 2.5 to 5.0 mg/mL and the bottle quickly sealed. Once the HDTMA dissolved, it was transferred to the syringe pump at 100 mL/minute, then pumped onto the soil column at a maximum flow rate of 0.25 mL/minute. Pumping continued until HDTMA was observed in the column effluent by the spectrometric method (see below). Pumping continued until HDTMA was reduced to a ppm level. Attempts to desorb HDTMA from soil with 0.05-M calcium sulfate, acetonitrile, or water were unsuccessful.

Soil samples for microbial counts as well as for naphthalene metabolism evaluation were obtained by inserting a common plastic drinking straw into the port and twisting it into the soil column for about 2.5 cm. The straw was withdrawn and the soil slug was pushed out with cotton Q-tips. The soil core was replaced with a small glass rod to maintain the soil column integrity. Samples for microbial studies were delivered to 20-mL glass liquid scintillation vials for further dilution and samples for analytical measurements were delivered to 2-mL polypropylene microfuge tubes. Samples obtained in this manner weighed between 0.7 and 1.5 grams. Core samples of the very top of the column were obtained by removing the tube fitting and inserting a drinking straw as above. Extracts for HPLC were prepared by vortexing the soil sample with 1.0 mL of acetonitrile followed by centrifuging for 4 minutes at 14,000g. The clarified acetonitrile was quickly transferred to a microinjection vial, capped tightly, and analyzed.

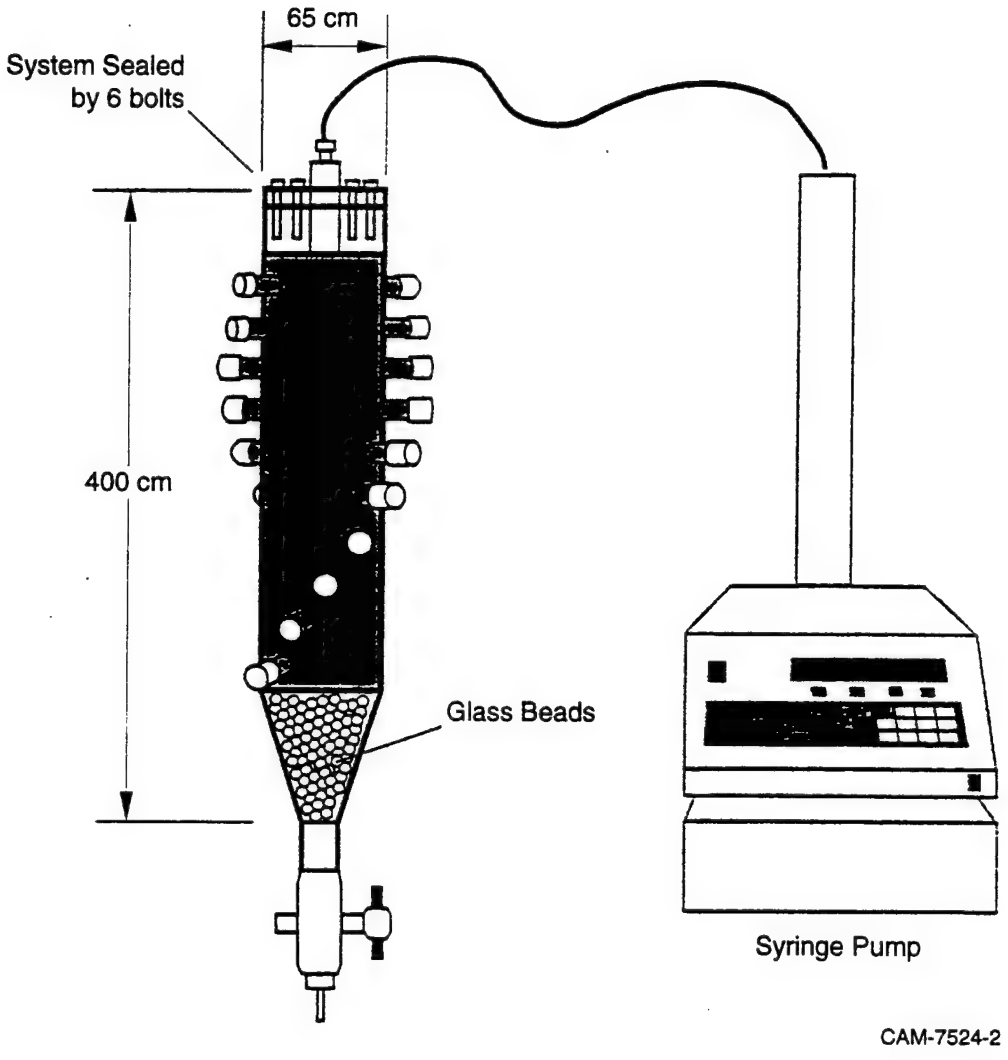


Figure 2. Soil column apparatus.

immediately. Because of the volatility of naphthalene, containers were kept frozen or chilled and minimal headspace was maintained in containers at all times.

3. Spectrophotometric Determination of HDTMA

The spectrophotometric procedure was a slight modification of the method for quaternary ammonium compounds described by Mitchell and Clark (1952). Briefly, in a 2-mL microfuge tube, 0.2 mL of sample is vigorously vortexed for one minute with 0.2 mL of 20% sodium carbonate, 0.2 mL of 0.8 mg bromphenol blue / mL of 30% dipotassium phosphate, and 1.0 mL of dichloroethane. The samples were centrifuged for 4 minutes at 14,000g and the upper excess dye reagent was aspirated away. The absorbance of the lower dichloroethane phase containing the complexed quaternary amine was determined at 600 nm.

Thin-layer chromatographic (TLC) investigations of the complex in dichloroethane were performed using Whatman silica gel 60A LK6DF with acetone or acetone containing 10% water as the mobile phase. The complex appeared as blue bands on a white background.

SECTION III

RESULTS

A. SOIL EVALUATION

The results of the soil evaluation by Agvise Laboratories indicate that the soil is classified as a sandy loam once sieved through a 2 mm sieve. The CEC (6.0 meq/100 grams) was used as a guide to loading the soil with the HDTMA surfactant . Other physical and chemical properties of the soil are shown in Figure 1.

1. Soil Sorption Isotherms

Columbus AFB soil was exchanged with HDTMA and thoroughly washed. Based on the CEC (6.0 meq/100 grams), we calculated that the soil carbon content was raised from 1.7% to 3.3%. Six naphthalene concentrations ranging from 0.97 to 20.4 ppm formed a linear soil sorption isotherm as shown in Figure 3. The slope yielded a soil sorption partition coefficient of 165 µg/gram/µg/mL. For untreated soil, the partition coefficient was 90 µg/gram/µg/mL

A K_{oc} ($K_{oc} = K_p/f_{oc}$ where f_{oc} is the fraction of organic carbon in the soil) value of 5300 µg/gram/µg/mL was calculated for the Columbus soil as a method to verify the effect of increased carbon loading. The K_{oc} for HDTMA-treated soil was calculated to be 5000 µg/gram/µg/mL, indicating that the estimate for HDTMA loading was approximately correct.

B. HDTMA SOIL COLUMN TOXICITY STUDY

Four soil columns were prepared and treated with 0%, 20%, 40%, and 80% of the CEC with HDTMA by gravity flow. The columns were thoroughly washed and extruded into four equivalent sections by weight. Each section was evaluated for microbial counts to estimate the effect of surfactant on microbial populations as a function of position and concentration in the soil column. The results are shown in Table 1.

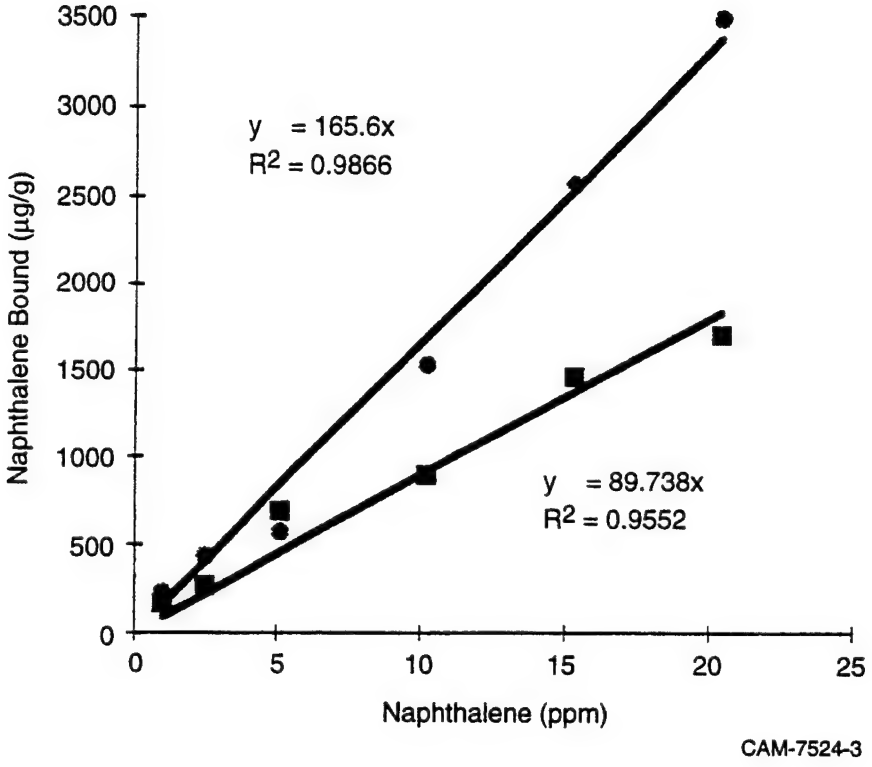


Figure 3. Sorption isotherms for naphthalene in HDTMA-treated and untreated Columbus aquifer soil.

TABLE 1. MICROBIAL POPULATIONS AS A FUNCTION OF HDTMA CONCENTRATION AND POSITION.

% HDTMA of CEC	Column Position*	Microbial Counts x 10^{-4}**
0%	1	9.33
0%	2	11.3
0%	3	19
0%	4	10
20%	1	0
20%	2	14
20%	3	7.0
20%	4	11.3
40%	1	0
40%	2	0.1
40%	3	13
40%	4	7.3
80%	1	0
80%	2	0.2
80%	3	0.4
80%	4	5.3

*1 = Top of Column; 4 = Bottom of Column
** = Colony-forming units/gram

The results of this study indicate the pronounced toxic effect of HDTMA on soil microbial populations. However, even at HDTMA concentrations at 80% of the CEC, microbial populations do survive below the top section. Thus, by controlling the HDTMA application rate with respect to the CEC, microbial populations will persist in the soil in areas where the CEC has not been exceeded.

C. SOIL COLUMN STUDY

1. Soil Column 1

The design and application of the soil column provided a microcosm environment where the loss and movement of a test chemical could be monitored under anaerobic and aerobic conditions and the viability of microorganisms determined. In the initial design, constant flow was found to be variable in soil packed columns. Different packing densities were tried, however, eventual shrinkage occurred, leading to high column back pressure (as measured by the Isco syringe pump). We found that the soil could be diluted with 30-mesh coarse sand (Mallinckrodt Chemical Co.) at a 1:2 ratio. The addition of sand improved the packing performance and reduced the column shrinkage dramatically. Column flows were generated at 150-250 μL/minute with pressures below 60 psig.

The column was packed (≈700 grams) and the pressure and flow rate were established with deoxygenated water. In the first experiment, HDTMA was prepared at 5.0 mg/mL in deoxygenated water and pumped through the column. Effluent concentration was monitored (Figure 4) until stabilization of a downward HDTMA concentration trend was achieved. Naphthalene was added (1071 μg in 50 mL water) and pumping was continued with deoxygenated water. After 700 mL was passed through the column, core samples were taken from Sampling Ports 1, 3, 5, 7, and 9 to evaluate the sorption and distribution of naphthalene in the soil and the soil column. Naphthalene was found in Samples 1, 3, and 5 at 10.5, 2.0, and 0.6 μg/gram, respectively. No naphthalene was found in Samples 7 and 9, nor were any naphthalene transformation products observed in any samples by high-performance liquid chromatographic (HPLC) analysis. These data suggest that we have generated and maintained an anaerobic system and that naphthalene has readily sorbed to the surfactant-bound soil.

Aerated water (600 mL) was passed through the column to activate aerobic populations and to initiate biodegradation. Soil samples were collected from Sampling Ports 2, 4, 6, 8, and 10, weighed, extracted with acetonitrile, and analyzed using HPLC. No naphthalene or metabolites were observed in Samples 2, 4, or 6, however, "metabolites" were evident in Sample 8 along with trace amounts (0.05 ppm) of naphthalene. Sample 10 showed minor amounts of metabolites and, again, trace amounts of naphthalene. Several of the metabolites coeluted with the reference standards, salicylaldehyde and 1,2-dihydroxynaphthalene. However, evaluation of the metabolite's UV spectra using a photodiode array (PDA) detector could not confirm the identifications due to slight differences in the absorption bandwidths even though the absorption maxima were nearly identical. The HPLC and UV spectra of the reference standards and the metabolites appear in Figures 5 and 6. We attempted to obtain mass spectral information using liquid chromatography/mass spectrometry (LC/MS) with thermospray but no ionization of the metabolites was observed to provide spectra. Later, we identified these "metabolites" as contaminants in Sample 8. It appears that naphthalene tends to move upon aeration, however, metabolism could not be confirmed. The loss of naphthalene from the soil is curious and its movement to the lower sections of the soil column provide evidence that aeration is having a major impact on the loss and movement of naphthalene in soil.

Samples collected from the opposite sides of Sampling Ports 2, 4, 6, 8, and 10 were evaluated for microbial populations. The number of colony-forming units (CFU) appears in Table 2.

TABLE 2. COLONY-FORMING UNITS/GRAM FOUND IN AEROBIC SOIL COLUMN SAMPLES.

SAMPLE No.	CFU/gram
2	1.9×10^5
4	1.9×10^5
6	1.2×10^5
8	2.3×10^4
10	6.7×10^4

Although some decrease in microbial populations in the sections 8 and 10 where "metabolites" were observed, the decrease may be the result of residual unbound HDTMA. The CFUs observed agree well with the values previously observed in soil when corrected for the soil dilution with sand. These results indicate the viability of microorganisms in a surfactant-

HDTMA IN COLUMN EFFLUENT USING WATER TO WASH COLUMN

Date	HDTMA (ppm)	
11/3–11/4	32	
11/4–11/5	36	
11/5–11/6	38	
11/6–11/6	41	
1/6–11/7[a]	44	
11/7–11/8	46	
11/8–11/8	42	
11/8–11/9[b]	88	
11/9	36	
11/10	63	
11/11	43	
11/12	27	
11/13	17	
11/14	25	
11/15	16	
11/16	17	
11/17	12	(naphthalene added this day)
11/18	14	
11/20	10	

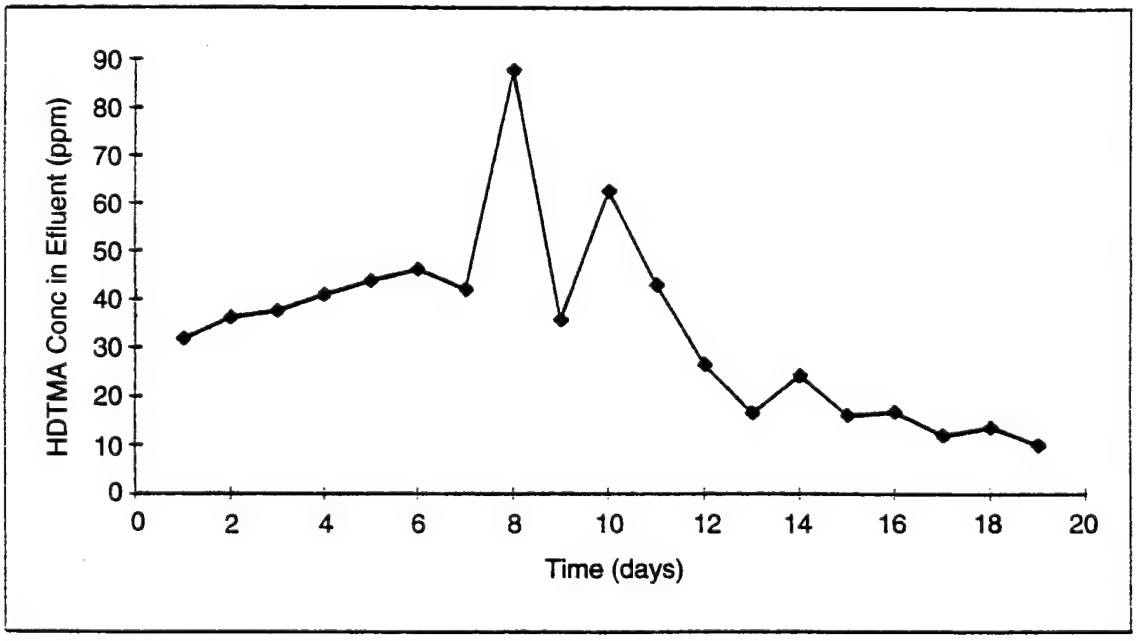


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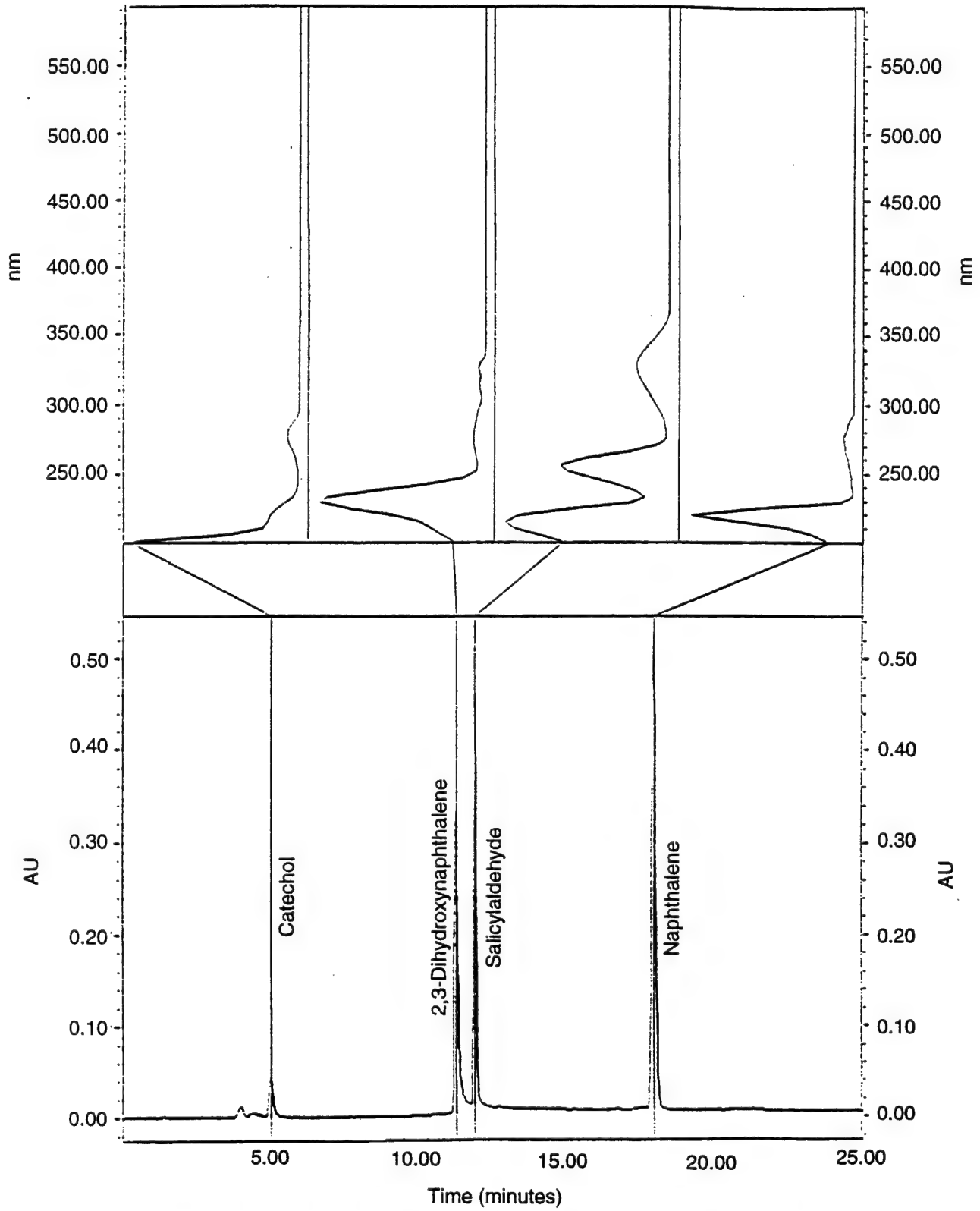
Figure 4. HDTMA concentration in Soil Column 1 effleunt.



Millennium PDA Spectrum Index Plot – SampleName Test Inject, PDA_220.0nm – PDA 220.0 nm

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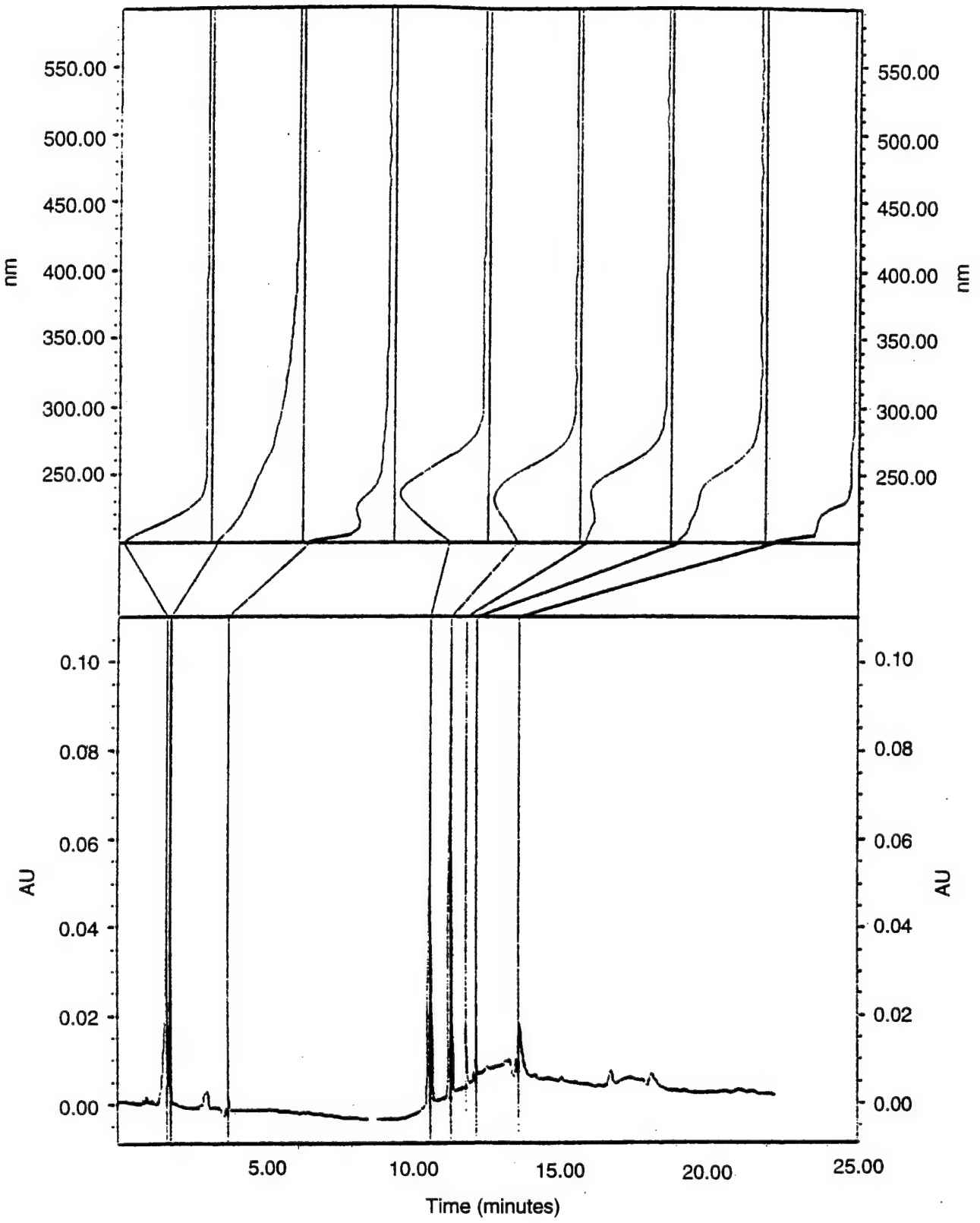
Figure 5. HPLC profile and PDA spectra of naphthalene and selected naphthalene metabolites.



Millennium PDA Spectrum Index Plot – SampleName Sample 8, PDA_220.0nm – PDA 220.0 nm

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Figure 6. HPLC profile and PDA spectra of Sample 8 "Metabolites."

treated soil. It appears that microbes can recover from surfactant-treated soil and become able to potentially degrade sorbed pollutants.

2. Soil Column 2

A new column was packed and washed with a higher flow rate of water (0.250 mL/minute). Little compression was observed and no fines were extruded. The higher back pressure (58 psig) has apparently compacted the fine particles to immobilize them. HDTMA was added to the column (2.5 mg/mL) and the column was washed with deoxygenated water (3000 mL) to a low HDTMA residue background (2.0 ppm, Figure 7) in the effluent flowing at 0.150 mL/minute. Naphthalene (1110 μg) was applied to the column void volume (≈ 54 mL) and 254 mL of deoxygenated water was passed through the column. Sampling of the top of the column and the Sampling Ports at Positions 1, 3, 5, 7, and 9 indicated that naphthalene was bound at the top of the column with only trace amounts observed in the Sampling Port 1 position (≈ 2 cm below the surface). However, components appeared in the HPLC profile from Sampling Ports 7 and 9 that might be related to naphthalene products based on their UV spectra (Figure 8) but they do not correspond with available standards. The long wavelength absorption of the component eluting at 12.093 minutes bears some resemblance to salicylaldehyde spectrum although the spectra are far from agreement (Figure 9). Initially, we thought that some fungal species may survive the HDTMA treatment due to their cellular wall components, however, none of the spectra of fungal metabolites (naphthols or naphthoquinones) matched with those observed.

The deoxygenated water was removed from the pump and replaced with oxygenated water. Pumping was initiated at 0.150 mL/minute. After 255 mL had been collected, the top of the soil and Sampling Ports 2, 4, 6, 8, and 10 were sampled for soil analysis and microbial counts. An additional 358 mL of oxygenated water was pumped through the column and sampling was again performed from the top and Sampling Port 1. An additional 460 mL was pumped and sampling was again performed at the top and Sampling Ports 2 and 3, and the next day from Sampling Ports 4, 5, and 6. The amount of naphthalene found at each port is shown in Table 3.

TABLE 3. DISTRIBUTION OF NAPHTHALENE (PPM) FOUND IN ANAEROBIC AND AEROBIC SAMPLES AND CFUs OBSERVED AFTER AERATION.

Port	Anaerobic (254 mL)	Aerobic (255 mL)	Aerobic (358 mL)	Aerobic (460 mL)	Aerobic (0 mL)	Microbial Counts
Top	5.4	3.1	1.33	0.16	NT	
1	1.4	NT	NT	NT	NT	
2	NT	0	0.83	0.87	NT	4.2×10^6
3	0.4	NT	NT	1.87	NT	
4	NT	0	NT	NT	0.72	2.0×10^6
5	0	NT	NT	NT	2.36	
6	NT	0	NT	NT	1.24	2.8×10^6
7	0	NT	NT	NT	NT	
8	NT	0	NT	NT	NT	2.3×10^6
9	0	NT	NT	NT	NT	
10	NT	0	NT	NT	NT	4.0×10^5

NT = Not Tested

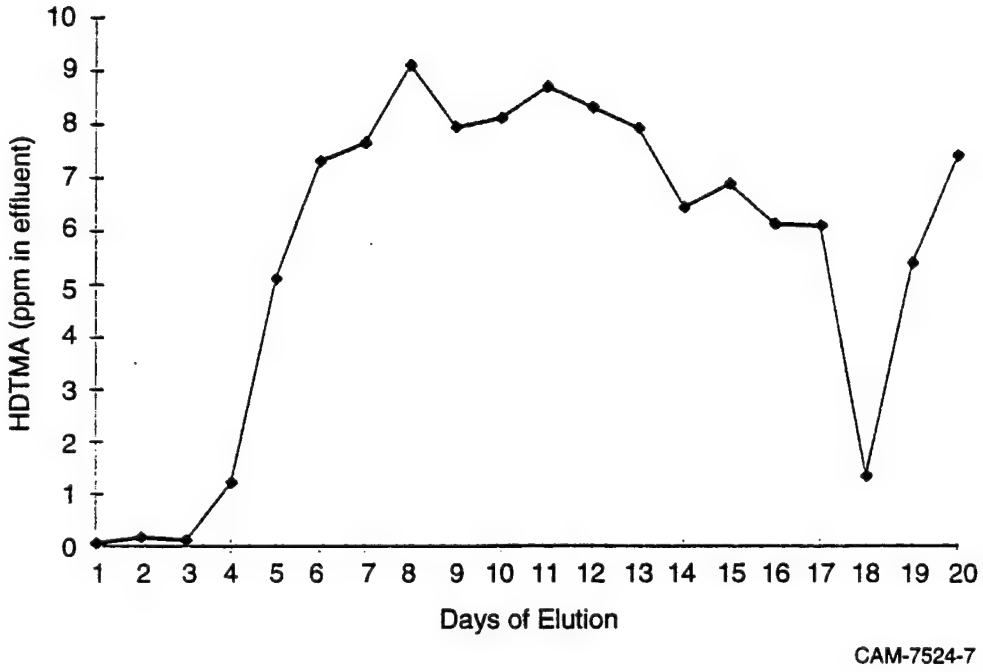
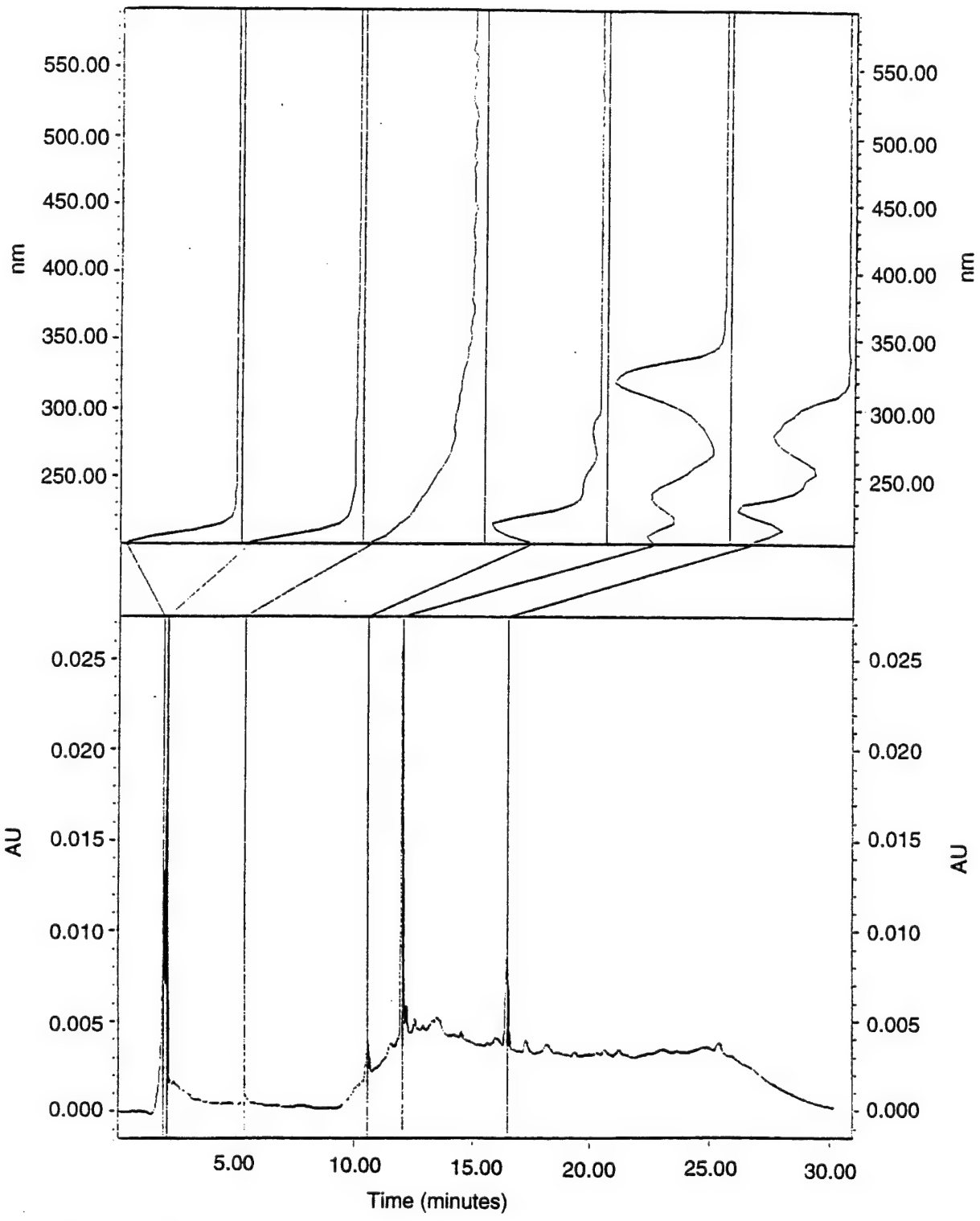


Figure 7. HDTMA concentration found in Soil Column 2 effleunt.



Millennium PDA Spectrum Index Plot – SampleName Anaerobic #9, PDA_230.0nm – PDA 230.0 nm

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Figure 8. HPLC profile and PDA spectra of Sample Port 9 soil under anaerobic conditions.

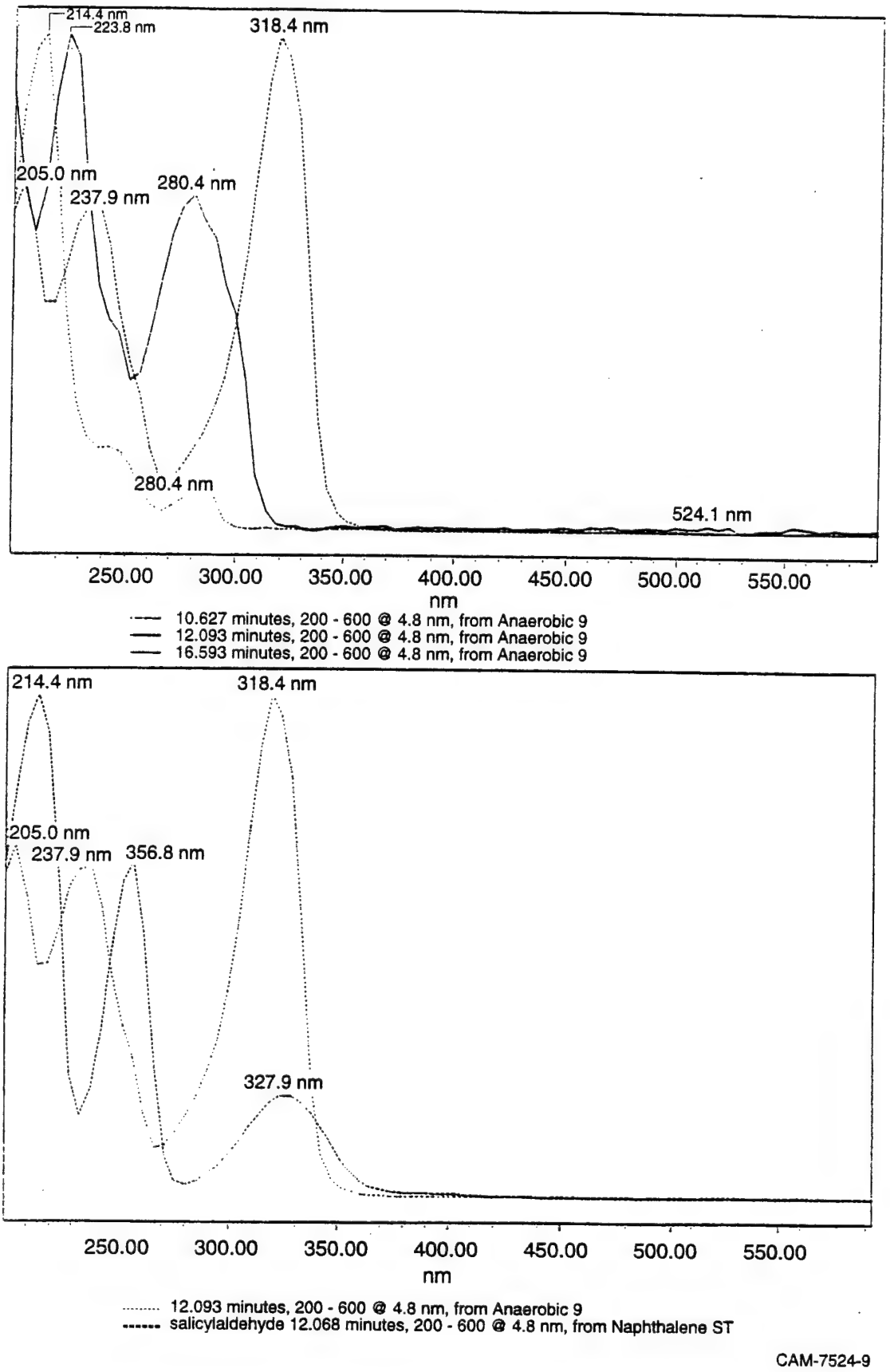
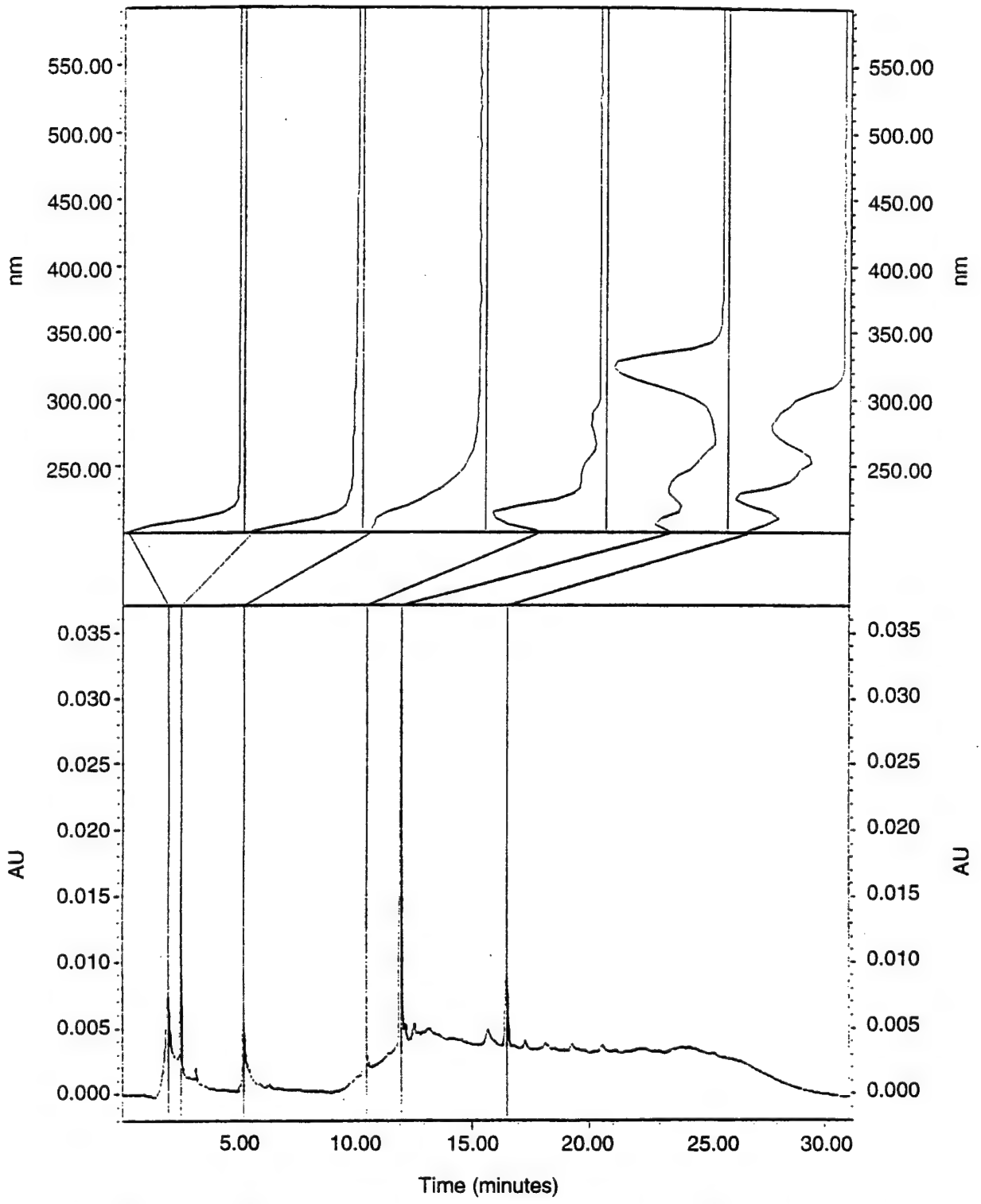


Figure 9. UV spectra of components observed in Anaerobic 9 soil (top) and a comparison of the UV spectra of the 12.093 component with salicylaldehyde (12.063 min).

The products observed in Sampling Port 9 under anaerobic conditions were also found in Sampling Port 10 under aerobic conditions (Figure 10). These results suggest that these products are not related to naphthalene but result from an inhomogeneity in the soil (although smaller amounts of these products were observed in Sampling Port 6 soil samples). The results indicate that naphthalene is primarily bound to the top of the column in the presence of deoxygenated water. When aerated water is added, naphthalene begins to disappear, however, no unique naphthalene metabolites were observed by HPLC relative to our available standards.

Continued aeration showed that naphthalene becomes lower in concentration at the head of the column but starts to move as a band down the column. While microbial populations of aerobes are significant, we are not sure why naphthalene should begin to migrate unless the surfactant is undergoing degradation.

The column effluent after aeration was analyzed for the surfactant and an increase in surfactant concentration was observed (Figure 11). Since the method of surfactant is nonspecific, the surfactant-BPB complex was analyzed by thin layer chromatography (TLC) (Figure 12). The complex is viewed visually as a blue-colored complex. The HDTMA complex migrated rapidly ($r_f = 0.68$) on silica gel with acetone as the mobile phase. The aerated column effluent complex hardly moved ($r_f = 0.10$). When water (10% in acetone) was added to the mobile phase, both complexes migrated with nearly equivalent r_f values (0.68 vs. 0.73). These data suggest a significant change in the surfactant composition upon the application of aerated water.



Millennium PDA Spectrum Index Plot – SampleName Aerobic #10, PDA_230.0nm – PDA 230.0 nm

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Figure 10. HPLC profile and PDA spectal profile of Sample Port 10 soil under aerobic conditions.

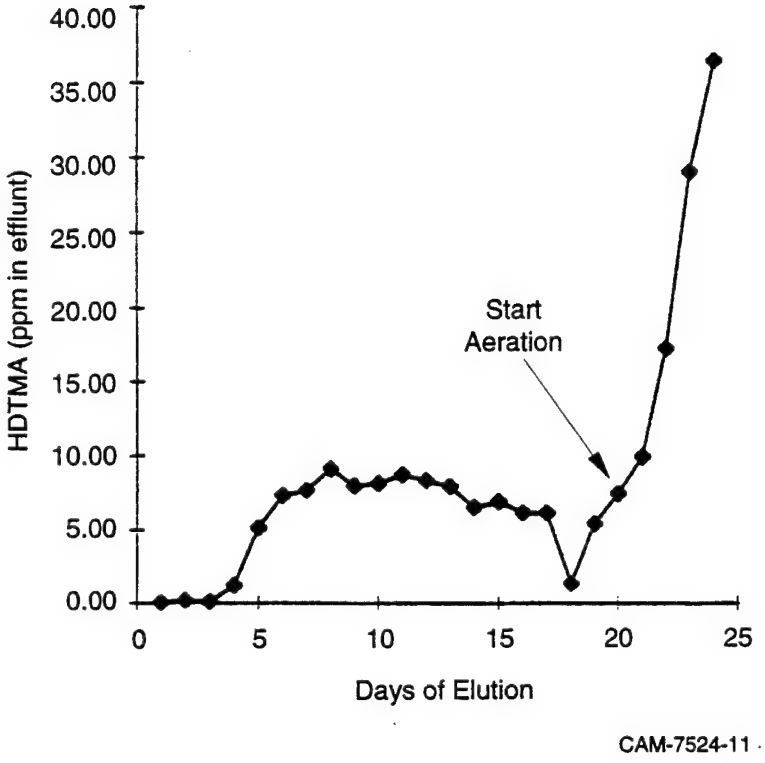


Figure 11. HDTMA concentration profile in the effluent under anaerobic and aerobic conditions.

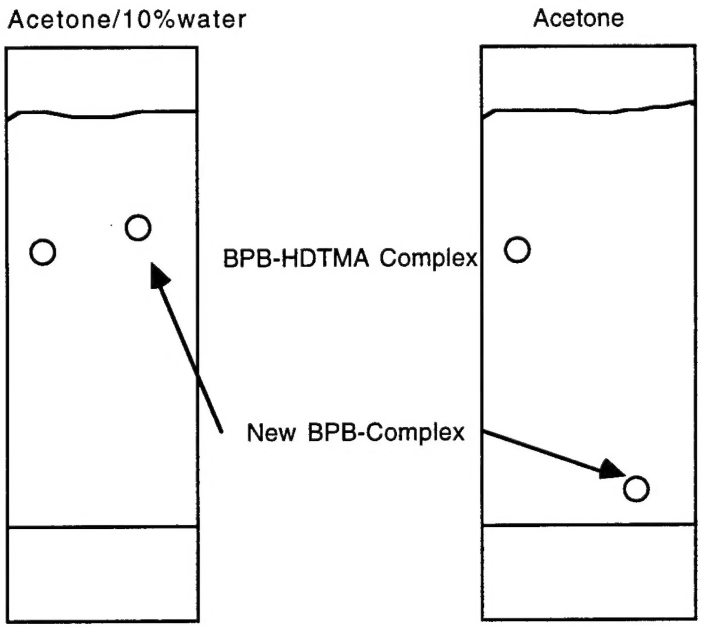


Figure 12. TLC Profile of Surfactant-BPB Complex After Exposure to Anaerobic (Left) and Aerobic Conditions (Right) Using Two Migration Solvents.

SECTION IV

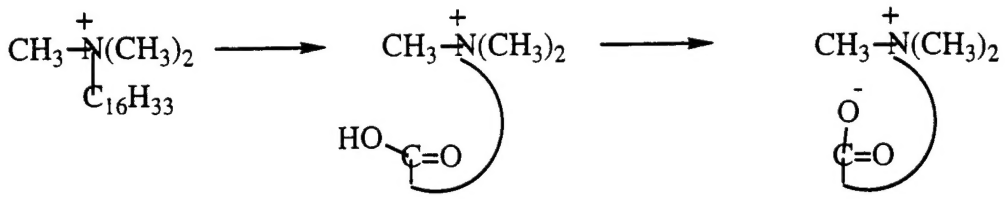
CONCLUSIONS

The results of this investigation support several conclusions reached by other investigators and provide new evidence regarding the potential behavior of naphthalene in an actual surfactant-treated soil environment. First, the addition of naphthalene to HDTMA-treated soil will increase its binding capacity to the soil, and therefore, it will retard its movement to ground water. Second, free HDTMA is toxic to soil microbial populations; however, soil microbial populations recover quickly to normal levels once the HDTMA is bound to the soil. Upon aeration, soil microbial populations appear to adapt to HDTMA at a faster rate than they do for naphthalene. This causes a breakdown in HDTMA, which in turn, allows naphthalene to migrate through the soil column.

The lack of naphthalene metabolism may be the result of an insufficient adaptation period for the indigenous microorganisms in Columbus aquifer soil. Mineralization half-life rates of 17 days have been found for adapted microorganisms (Heitkamp et al., 1987) in soil/water microcosms, whereas Bauer and Capone (1980) observed a 21-day period to mineralize 32% of naphthalene in an intertidal marine sediment. These rates suggest that intermediate products should appear readily when adapted microorganisms are present. Heitkamp et al. (1987) identified naphthalene metabolites within seven days after the application of naphthalene to sediment-water microcosms, whereas fungal metabolism by Cunningham *elegans* produced metabolites within 24 hours (Cernigilia and Gibson, 1977).

HDTMA utilization by bacteria has been shown by Nye et al. (1994). In their studies, 32% of the added ^{14}C-label was converted to CO_2 in 3 days in soil slurry mixtures. Our study confirms HDTMA utilization; however, the HDTMA utilized is in the bound form since extensive column washing was performed before the addition of oxygenated water. The HDTMA breakdown was observed in the column effluent by an increase in the concentration of quaternary ammonium compounds, suggesting that the microbial metabolism decreases the surfactant's binding capacity to the CEC sites within the soil matrix. The TLC behavior of the complexes formed with BPB on silica gel suggests that much more polar surfactants have been formed upon the addition of oxygenated water. HDTMA breakdown is supported by the migratory behavior of naphthalene in the soil matrix.

It is interesting to speculate on the nature of the intermediates formed since the tetraalkylammonium moiety must still be intact to produce a positive BPB complex. Also, the modified surfactant is no longer tightly bound to the soil's CEC sites. In the work reported by Nye et al.(1994), HDTMA was labeled at the terminal C16 position and eventually was collected as $^{14}CO_2$. This suggests that possibly a zwitterionic species is produced from oxidized surfactant which decreases its binding capacity by intramolecular association as shown below.



A carboxylic functionality would be a precursor to carbon dioxide formation and it would also increase the aqueous solubility of the oxidized surfactant and increase it's mobility through the soil column. The TLC mobility on silica gel could also be explained by such a moiety. While this type of explanation is consistent with the observed results, further investigations are required to confirm these findings.

The findings of this investigation suggest we must be careful of the impact of biologically modified surfactant on ground water supplies when surfactant-modified soil technology is applied, especially on soils where pollutant-adapted microbial populations do not recover fast enough to compete with the use of surfactant.

SECTION V

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